HARVESTING OF MICROALGAE *Botryococcus* sp. THROUGH PHYCOREMEDIATION OF BATHROOM GREYWATER

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A thesis submitted in
Fulfillment of the requirement for the Award of the
Degree of Master of Civil and Environmental Engineering

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NOVEMBER 2016

DEDICATION

This work is dedicated to three men in my life, my beloved dad Air Commodore Atiku Umar Jalingo (Rtd), my baby boy Ahmad Rayyan and my dear husband Anwar Ahmed, i love you all.

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my supervisor Dr Radin Maya Saphira Radin Mohamed for the continuous support of my master's study, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor and mentor for my master's study.

My warm appreciation goes to my beloved husband Anwaruddin Ahmed, he was not just a husband but he became a father, a friend, a confidant, and a source of encouragement. Thank you so much for your resources, time, energy and support spent to make my programme a success, may Allah (SWT) grant you jannatul firdaus. My sincere thanks also goes to Dr Adel Algeethi for his guidance and technical corrections on my thesis and review paper. I also would like to thank the technicians of Environmental Engineering, Wastewater and Analytical Laboratory, Faculty of Civil and Environmental Engineering, Universiti Tun Hussien Onn Malaysia for their assistances in performing the laboratory work throughout the process of compiling this thesis.

My heartfelt gratitude goes to my dad Air commodore Atiku Umar (Rtd) and my mum Mrs Maryam Atiku, thank you for always praying, loving, providing and always being there for me as lovely parents, may Allah bless you. Next to my lovely in-law Alhaji Ahmed Usman Wurochekke and family for their endless prayers and support, I am so lucky to be your daughter in-law. Also my profound gratitude goes to my second family in Malaysia Group captain Bakari & family, thank you for your love and encouragement. Lastly, I will like to say a big thank you to my friends, brothers, sisters, nieces and nephews, I love you all.

ABSTRACT

Malaysia has been experiencing water pollution crisis over a decade and one of the causes is direct discharge of greywater into the drains. Therefore, phycoremediation of greywater especially from bathroom sources with microalgae is proposed. The objectives of this research are to measure the efficiency of *Botryococcus* sp. in assimilating pollutant load, to optimize the flocculation process and investigate the interactive effects of experimental factors through Response Surface Methodology (RSM) design expert software. *Botryococcus* sp. was inoculated in bathroom greywater with 4.5 x 10^6 cell/mL for phycoremediation process. Microalgae *Botryococcus* sp. grown in bathroom greywater from House A, House B and House C and tested for reduction of pollutant. Results shown that the highest reduction for COD was at House A with 87 %, BOD_5 was reduced to 96 % at House C which is the highest reduction, NH_4^+ highest was reduced at House A with 98 % and PO_4^{3-} highest reduction occurred at House C with 88 % on the 21^{st} day of phycoremediation respectively. Whereas, *Moringa oleifera* and *Strychnos poatatorum* were used in harvesting *Botryococcus* sp. in bathroom greywater via flocculation technique. A central composite design, which is the standard design of response surface methodology (RSM), was used to evaluate the effects and interactions of three factors, i.e. coagulant dosage, settling time and pH on the harvesting efficiency. Lastly, the biomass recovery was conducted via optical density to calculate the biomass recovery for *M. oleifera* and *S. potatorum*. The optimal conditions obtained from the compromise of one desirable responses, turbidity was at coagulant dosage of 10 mg /L, settling time of 120 min, and pH 9 respectively. The biomass recovery percentage for *Botryococcus* sp. by using *Moringa oleifera* and *Strychnos potatorum* were 97 % and 81 % accordingly. Therefore, this study proved that the cultivation of microalgae in bathroom greywater was successful in reducing the amount of pollutant tested in this research.

ABSTRAK

Malaysia telah mengalami krisis pencemaran air lebih satu dekad dan salah satu punca ialah pelepasan langsung air sisa kelabu ke dalam longkang. Oleh itu, kaedah menggunakan *phycoremediation* daripada air sisa kelabu terutamanya dari sumber mandi dengan mikroalga telah dilakukan. Objektif kajian ini adalah untuk mengukur kecekapan *Botryococcus* sp. dalam penerapan beban pencemar, untuk mengoptimumkan proses penuaian dan menyiasat kesan interaktif faktor eksperimen melalui *Response Surface Methodology* (RSM) perisian pakar reka bentuk. *Botryococcus* sp. telah ditambah dengan air sisa kelabu bilik mandi dengan 4.5 x 10^6 sel / mL semasa proses *phycoremediation*. Mikroalga *Botryococcus* sp. dicampurkan dengan air sisa kelabu bilik mandi dari Rumah A, B dan C dan diuji bagi mengetahui pengurangan bahan pencemar. Keputusan kajian menunjukkan bahawa pengurangan tertinggi bagi COD adalah di Rumah A dengan 87%, BOD_5 telah dikurangkan kepada 96% di Rumah C yang merupakan penurunan tertinggi, NH_4^+ tertinggi dikurangkan di Rumah A dengan 98% dan PO_4^{3-} pengurangan tertinggi berlaku di Rumah C dengan masing-masing 88% pada hari ke-21 proses *phycoremediation*. Manakala, *Moringa oleifera* dan *Strychnos poatatorum* telah digunakan dalam menuai Botryococcus sp. dan air sisa kelabu bilik mandi melalui teknik pemberbukuan. Satu reka bentuk komposit pusat, yang merupakan reka bentuk standard metodologi gerak balas permukaan (RSM), telah digunakan untuk menilai kesan dan interaksi tiga faktor, iaitu koagulan dos, masa penetapan dan pH pada kecekapan menuai. Akhir sekali, pengiraan biomass itu dijalankan melalui ketumpatan optik untuk mengira perolehan biomas untuk *M. oleifera* dan *S. potatorum*. Syarat-syarat yang optimum diperolehi menunjukkan kekeruhan adalah pada dos koagulan 10 mg / L, masing-masing masa penetapan 120 min, dan pH 9. Biojisim pemulihan peratusan bagi *Botryococcus* sp. dengan menggunakan *Moringa oleifera* dan *Strychnos potatorum* adalah 97% dan 81%. Oleh itu, kajian ini membuktikan bahawa rawatan dan penuaian alga menggunakan air sisa kelabu bilik mandi telah berjaya mengurangkan jumlah pencemar diuji dalam kajian ini.

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LIST OF ABBREVIATIONS

BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
TSS	-	Total Suspended Solids
EC	-	Electrical Conductivity
NH_4^+	-	Ammonium
NO_3^-	-	Nitrate
OP_4^{3-}	-	Orthophosphate
OD	-	Optical Density
CO_2	-	Carbondioxide
DHA	-	Docosahexaenoic Acid
GHG	-	Green House Gases
Mg	-	Magnesium
Ca	-	Calcium
TN	-	Total Nitrogen
TP	-	Total Phosphorus
DNA	-	Dioxoribonucleic Acid
RNA	-	Ribonucleic Acid
ATP	-	Adesonine triphosphate
RSM	-	Response Surface Methodology
NPs	-	Nanoparticles
PDDA	-	Polyelectrolyte polydiallyldimethyl Ammonium Chloride
DAF	-	Dissolved Air Flotation
CTAB	-	Cationic N-cetyl-N-N-N trimethyl Ammonium
Rpm	-	Rounds per minute
BGW	-	Bathroom greywater
NaCl	-	Sodium Chloride
UTHM	-	Universiti Tun Hussein Onn Malaysia
PET	-	Polyethylene terepthalate
APHA	-	American public health association

CCD	-	Central Composite Design
SMBR	-	Submerged membrane bioreactor
FSTPI	-	Faculti Sains, Teknology dan Pembangunan Insan

LIST OF PUBLICATIONS

1) Improvement of Bathroom Greywater Quality after Phycoremediation with Microalgae *Botryococcus* sp. presented at the International Conference on Environmental Forensics 2015 (IENFORCE 2015), Putrajaya Marriot Hotel, Malaysia, 19-20 August 2015.
2) Bathroom Greywater Bioremediation by Microalgae *Botryococcus* sp. (ICSESS 2016) presented at the 2nd International Conference on Science, Engineering and the Social Sciences, 29 May - 1 June 2016, Universiti Teknologi Malaysia, Johor Bahru, Malaysia. Organized by UTM International and UTHM.

Journal:

1) Hauwa Atiku Majidda, Radin Maya Saphira Radin Mohamed, Al-Gheethi AA, Anwaruddin Ahmed Wurochekke & Amir Hashim Mohd Kassim. Harvesting Microalgae Biomass from the Phycoremediation Process of Greywater. Environmental Science and Pollution Research, Vol, 23 (16) Springer (ISI, Impact Factor= 2.8, Q1).
2) Anwaruddin Ahmed Wurochekke, Radin Maya Saphira Radin Mohamed, Al-Gheethi AA, Hauwa Atiku Majidda & Amir Hashim Mohd Kassim. Household Greywater Treatment Methods using Natural Materials and their Hybrid System, Journal of Water and Health, Vol, 14 (5) 2016, wh2016054; DOI: 10.2166/wh.2016.054), IWA Publishing. (ISI, Impact Factor= 1.45, Q2).
3) Hauwa Atiku Majidda, Radin Maya Saphira Radin Mohamed, Al-Gheethi AA, Anwaruddin Ahmed Wurochekke & Amir Hashim Mohd Kassim. Optimizing *Botryococcus* sp. Biomass Harvesting from Greywater by Natural Coagulants. Waste and Biomass Valorization (ISI, Impact Factor= 0.9), Under Review.

CHAPTER 1

INTRODUCTION

1.1 Background

Wastewater can be classified into domestic wastewater, industrial wastewater and municipal wastewater. Wastewater contains contaminants that have harmful impact on the environment if not controlled in terms of removal. This is because wastewater containing nitrogen and phosphorus affects the natural ecosystem thereby causing eutrophication. Greywater is generated from domestic wastewater which refers to the untreated wastewater that is collected from household activities such as baths, showers, washing machines, laundry troughs, dishwashers and kitchen sinks, except toilet wastes (Mohamed *et al.*, 2014).

The raw bathroom greywater creates pond and small pools that supply a good environment for pathogenic bacteria to generate and develop into a proliferation environment for insect pests, poses a potential risk to human health. These harmful effects from bathroom greywater are likely to be more severe in slums where sanitation is inadequate (Katukiza *et al.*, 2014). In Indonesia, the main sources of the water pollution are domestic wastewater which contributes (around 60%) and industrial wastewater (around 40%) discharged directly into the water bodies without treatment (Prihandrijanti & Firdayati, 2011).

The quality of greywater varies depending on the source and use of greywater, geographical location, social habits, demographics and level of occupancy (Prathapar *et al.*, 2005). For instance, the average annual household greywater production in Arizona is estimated to be from 30000 to 40000 gallons (Al-Jayyousi, 2003). In Los Angeles, domestic greywater produces about 21 to 59 gallons per capita which is discharged untreated into the water bodies daily representing 53-81% of the total wastewater generated from residents. On a worldwide level, the decreasing availability of conservative water sources has increased the demand for high quality freshwater. This initiates led the individuals to think the alternative and sustainable solutions to manage this precious resource.

Water pollution is a global problem and there has been potential use of phycoremediation technology whereby the nutrients in greywater is used as feed for microalgae growth (Sharma *et al.*, 2014). The phycoremediation technique is useful in removing excess nutrients such as nitrogen, phosphorus from wastewater and also has ability to reduce various pollutants like BOD, COD, heavy metal, faecal coliform and E.coli. According to Gokulan *et al.*, (2013) *Botryococcus* sp. showed good response towards the treatment of greywater from mens hostel, nitrogen and phosphorus were reduced from 14.21-0.31 ppm and 9.64-2.16 ppm considerably after phycoremediation.

The selection of harvesting methods has a significant impact on the reusability of recycled water, as it will affect the quality of water after the harvest. Harvesting of algae has been described by many researchers as the main challenge in the production of microalgae biomass (Hamawand *et al.*, 2014). Many different methods have been employed to harvest microalgal cells which includes centrifugation, organic and inorganic flocculants, polymer, membrane filtration technology and dissolved air flotation (Farooq *et al.*, 2015). Some techniques such as centrifugation are very energy demanding, whereas organic polymers like chitosan are costly. Cationic polymers also have been used, but they are not as effective in saline culture mediums. Other harvesting methods such as electrolytic flocculation and electro-flotation also have been effectively applied with low energy cost. More importantly for the purpose of reusing the medium, the choice of harvest method must not introduce substances that are toxic to the cells nor have negative impacts on biomass quality.

Optimization is usually carried out in conventional multifactor experiments by varying a particular factor while keeping all other factors fixed at a specific set of conditions. It is not only time-consuming, but usually unable of reaching the true optimum due to disregarding the contacts among variables. In contrast, the response surface method (RSM) has been projected to determine the influences of individual factors and their interactive influences (Wang *et al.*, (2007).

The RSM is a statistical method for designing experiments, building models, evaluating the effects of numerous factors, and searching optimum conditions for desirable responses. Through RSM, the relations of possible influencing parameters on treatment efficiency can be evaluated with a limited number of designed experiments. RSM is commonly used in various fields, e.g. coagulation–flocculation process for a paper-recycling wastewater treatment (Wang *et al.,* 2007), harvesting *Chlorella vulgaris* using a novel bio-source *Strychnos potatorum* (Abdul Razack *et al.,* 2015), Coagulation and flocculation process for dye removal using sludge from water treatment plant (Sadri *et al.*, 2010), coagulation-flocculation process for pulp mill wastewater treatment (Wang *et al.*, 2011).

Although there has been significant work on algal wastewater treatment, however, some attempts have not been made towards harvesting microalgae *Botryococcus* sp. from phycoremediation of bathroom greywater. Hence, this present study is focused on the phycoremediation of bathroom greywater with microalgae *Botryococcus* sp. to reduce the amount of pollutant present in bathroom greywater and to harvest microalgae biomass after phycoremediation with different flocculant aid *Moringa oleifera* and *Strynchnos potatorum*. The microalgae biomass has potential to be used in producing organic fish feed, cosmetics, human nutrition, animal feed and biofuel for future application.

1.2 Problem Statement

In many parts of the world, water scarcity is one of the most significant challenges to human health and environmental integrity. As the world's population grows and prosperity spreads, water demands increase and develop without the possibility for an increase in supply. The increasing demand on this limited and precious resource has inspired creative approach for freshwater management, including innovative techniques for greywater phycoremediation. Choul-gyun, (2002) comprehend that wastewater treatment using algae is more environmental friendly and economical as it does not entail any complicated system and chemical. In many part of village houses in Malaysia, such as Parit Raja Johor malaysia, greywater does not receive any adequate treatment before it is discharged into drains, as a result, the receiving streams and rivers have become polluted.

This resulted in an unhealthy environment for humans or animals, which can cause dangerous diseases. Environmental pollution problems also occurred as extreme amounts of bathroom greywater from growing populations overloaded nearby water bodies. Therefore, it is important to treat bathroom greywater to reduce the pollutant load before discharge into the drain.

Aluminum Sulfate commonly known as Alum, is proven to be an effective flocculant for some species of microalgae, flocculation by metal chelate such as this is positively improper if the harvested biomass is used for aquaculture purpose, animal feed or organic fertilizer. It was reported that the main component of Alum and Acrylamide could lead to human health implications, such as involvement in Alzheimer’s disease and the cause of cancers. Hence, the use of natural flocculant would be an option in overcoming this possibility.

1.3 Aim and Objectives of study

The aim of the study is to investigate the efficiency of natural flocculants in harvesting of *Botryococcus* sp. through phycoremediation of bathroom greywater. Based on the aim of the study, three objectives have been formulated;

i. To evaluate the efficiency of phycoremediation in treating bathroom greywater with microalgae *Botryococcus* sp.

ii. To optimize the harvesting of *Botryococcus* sp.from the phycoremediation through flocculation method using *Moringa oleifera* and *Strychnos potatorum*. using Response Surface Methods (RSM).

iii. To quantify the microalgae biomass from the harvesting process of *Botryococcus* sp.

1.4 Scope of Study

This study was carried out by using microalgae that was isolated from the forest of Endau Rompin, Johor, Malaysia. The isolates were transferred to the Faculty of Science and Human Development (FSTPI) laboratory University Tun Hussein Onn Malaysia (UTHM) for culturing. Microalgae *Botryococcus* sp. was used for phycoremediation of bathroom greywater. Meanwhile, the bathroom greywater was obtained from two houses in Parit Haji Rais and one house in Parit Haji Abdul Kadir, Batu Pahat, Malaysia. Parameters tested include Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), pH, Total Suspended Solid (TSS), Electrical conductivity (EC), Ammonium $\left(NH_4^+\right)$, Nitrate $\left(NO_3^-\right)$, and Orthophosphate (PO_4^{3-}).

Harvesting of microalgae biomass was conducted using flocculation technique with *M.oleifera* seed powder and *S. potatorum* seed powder. Design expert software version 7.0 was used for optimizing factors such as dosage, pH and settling time via Response Surface Method (RSM). Lastly, the harvested biomass was quantified by optical density (OD) and dried in oven at 60°C for 24 hr, the dried biomass was analyzed using Scanning Electron Micrographs (SEM).

1.5 Significance of Research

Malaysia is one of the developing countries with so many populations and as a result greywater is discharged directly into the rivers. This situation is of great concern for the government, community dwellers, operating companies, stakeholders, supervisory and regulatory agencies. Therefore, this study is to produce simple greywater treatment that will be used commercially for public. The phycoremediation technique can be used as low cost product and can still reduce the pollutants present in bathroom greywater which are discharged directly into the water bodies without treatment. Throughout this research, control of pollution at the house level can provide a vast potential to treat bathroom greywater with *Botryococcus* sp. with a net energy savings to the water system.

In addition, it can decrease the need for fresh water and reduce the amount of greywater that enters the drains and water bodies. The microalgae biomass harvested has the potential to be utilized in producing fish feeds, fertilizer, pharmaceuticals, biofuel and cosmetics.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discussed about the previous studies related to this research. Bathroom greywater characteristics and the treatments found in the literature are described. A review of phycoremediation, microalgae, response surface methodology, harvesting methods and drying method are presented.

2.2 Bathroom greywater

Bathroom greywater is generated from (bath, sink, and shower) which contribute about 59 per cent of the total usable greywater volume in a typical household (Loh & Coghlan, 2003). Bathroom greywater is contaminated with hair, soaps, shampoos, hair dyes, toothpaste, lint, nutrients, body fats, oils and cleaning products. It may also include some faecal contamination (and the associated pathogens) through body washing.

2.3 Characteristics of Bathroom Greywater

The characteristics of greywater depend on the number of household occupants, the age difference of the occupants, their lifestyle, water usage patterns, living standards, social and cultural habits (i.e. type of soaps, toothpastes, shampoos, detergents, etc). Bathroom greywater contains soaps, shampoos, toothpaste, body care products, shaving waste, skin, hair, body fats, lint, and traces of urine and faeces (Jefferson *et al*., 2004).

The strength of greywater normally is expressed in terms of pollution loads, which is determined from concentration of physical, chemical, and biological contents of the greywater. Knowledge of pollutants concentration is significant for selecting a particular treatment method for removal of pollutants that meets the limits of standard for state water disposal before final discharge into the drains. The strength of greywater is normally measured as mass per unit volume of wastewater and are expressed in milligrams per liter.

The greywater is physically polluted by particles of dirt, food, lint, sand etc. The water is polluted chemically by dissolved salts such as sodium, nitrogen, phosphate and chloride or by organic chemicals such as oil, fates, milk, soap and detergents which may provide food for micro-organism and plant growth. Micro-organism, many of which are pathogenic and contaminates greywater e.g faecal coliform, E.coli. Table 2.1 shows the characteristics of bathroom greywater and its related sources.

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